新規発根促進剤として有望なエストロジェン活性を持たない5、6−ジクロロインドール−3−酢酸と4−クロロインドール−3−酢酸

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5,6-Dichloroindole-3-acetic acid and 4-chloroindole-3-acetic acid, two potent candidates for new rooting promoters without estrogenic activity

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5,6-Dichloroindole-3-acetic acid (5,6-Cl₂-IAA, 1) and 4-chloroindole-3-acetic acid (4-Cl-lAA, 2), synthesized from the corresponding chlorinated indole compounds, showed strong rooting-promoting activity in black gram cuttings. 5,6-Cl₂-IAA was the most potent of all compounds examined. At a concentration of 5×10⁻⁵ M, its activity was 15 times higher than that of 4-(3-indole)butyric acid (IBA), an active ingredient in commercially available rooting promoters. The activity of 4-Cl-lAA was also four times higher than that of IBA at the same concentration. 5,6-Cl₂-IAA and 4-Cl-lAA had no estrogenic activity as measured using an estrogen receptor binding assay. © Pesticide Science Society of Japan

Keywords: rooting promoter, 5,6-dichloroindole-3-acetic acid, 4-chloroindole-3-acetic acid, estrogenic activity, endocrine-disrupting activity.

Introduction

Many compounds from a variety of chemical categories, including pesticides, food additives, and environmental contaminants, have adverse effects on aquatic animals and mammals, and by extension most likely on humans. Polychlorinated dibenzo-p-dioxins and biphenyls, plasticizers (bis(2-ethylhexyl)phthalate, dibutylphthalate, etc.), pesticides (DDT, atrazine, benomyl, etc.), food additives (di- and butylhydroxyanisole, morin, etc.), and resin materials (bisphenol A, alkylphenols) are well-known endocrine disruptors.¹⁻⁷ Many pesticides, in particular chlorinated insecticides, exert endocrine-disrupting activity.¹⁻⁹

We have been developing new rooting promoters for the mass production of saplings of important, useful and rare trees for the purposes of reforestation and preservation of endangered plant species. Seradix (Oxyberon, Hormidon) and Rooton (NAD, Transplanton), well-known commercially available rooting promoters, are used to make saplings from cuttings of flowers and ornamental plants as well as from trees; however, there are many cases in which it is difficult if not impossible to induce plant roots despite using the above rooting promoters at varying concentrations under diverse conditions. Indole-3-butyric acid (IBA) and 1-naphthaleneaceticamide (NAD), active ingredients of the rooting promoters, were natural and synthetic auxin, respectively, but 4-chloro-2-methylphenoxyacetic acid, a synthetic chlorin-containing auxin, has been reported to have an endocrine-disrupting effect,⁹ however, estrogenic activities of IBA and NAD have not been reported. For these reasons, it has been hoped for the development of potent new rooting promoters without endocrine-disrupting activity, so it is important that the estrogenic activity of newly developed rooting promoters with auxin activities should be checked, as well as their toxicities, before they are made commercially available.

The synthetic 5,6-dichloroindole-3-acetic acid (5,6-Cl₂-IAA, 1) is the most active of the known natural and synthetic auxins, as determined by *Avena* coleoptile elongation, inhibition of Chinese cabbage hypocotyl growth, mung bean hypocotyl swelling, and lateral root formation.¹¹ 4-Chloroindole-3-acetic acid (4-Cl-lAA, 2) and its ester also have strong auxin activities and significantly boost the root formation of *Serissa japonica* cuttings.¹¹ 4-Cl-lAA, the most active natural auxin, is isolated from immature seeds of plants belonging to the *Ficeae* tribe,¹²–²² some of which are consumed daily by humans, and from immature and mature scotch pine seeds. 5,6-Cl₂-IAA and 4-Cl-lAA will be potent candidates as active ingredients of new rooting promoters. We report here that these two compounds are highly potent candidates for new rooting promoters without estrogenic activity (Fig. 1).

![Chemical structures of 5,6-Cl₂-IAA and 4-Cl-lAA.](image-url)
Materials and Methods

**Chemicals.** 4-Cl-IAA was synthesized from 2-chloro-6-nitrotoluene using the procedure reported previously by Katayama.\(^1\) 5,6-Cl\(^{-2}\)-IAA was newly synthesized from 5,6-dichloroindole in a similar manner. 4-(3-Indole)butyric acid (IBA) and IAA were purchased from Kanto Kagaku Co., Ltd. (Tokyo, Japan) and Merck Co., respectively.

**Plant Materials.** Black gram (Vigna mungo (L.) Hepper; Sakata Seed Co., Japan) seeds, which had been stored at 5°C, were used in the subsequent rooting-promoting activity test. Black gram seeds were sowed into Akadama soil (Fujimi Gardening Materials Co., Shizuoka, Japan) and grown at 25°C in an incubator (Shimadzu BEC-II-250HUP) under a 16-h light (7,000lux)/8-h dark cycle for 9 days.

**Rooting-promoting activity test with black gram cuttings.** Seven-centimeter cuttings with purplish-red hypocotyls were cut from plants grown in an incubator (Shimadzu BEC-II-250HUP) under a 16-h light (7,000 lux)/8-h dark cycle for 2 weeks. The water was changed every 3 h. The soaked cuttings were washed thoroughly with 20 µl spreading agent (Dain; Sumika-Takeda Garden Chemicals. 4-CI-IAA was synthesized from 2

Results and Discussion

Among 10 dichloroindole-3-acetic acids, 5,6-Cl\(^{-2}\)-IAA has been shown to have the strongest activity in terms of elongating the *Avena* coleoptile, inhibiting Chinese cabbage hypocotyl growth, and inducing hypocotyl swelling and lateral root formation of intact black gram.\(^10\) 5,6-Cl\(^{-2}\)-IAA induced many short lateral roots on swollen and growth-inhibited hypocotyls at 1×10\(^{-6}\)-3×10\(^{-5}\) M in 3 days, but did not induce roots by the treatment of intact plants at the same concentration for 3 hr. In this study, 5,6-Cl\(^{-2}\)-IAA was the most effective substance for promoting root formation in black gram cuttings by treatment for 3 hr (Table 1). At a concentration of 5×10\(^{-5}\) M, its induced root number was 15 times more than that of IBA, the active ingredient in commercially available rooting agents, such as Seradix, Hormodin and Oxyberon. Black gram hypocotyl, with its large number of roots, looked like a test tube brush (Fig. 2). Further, many root primordia were induced on the upper portion of hypocotyls which had not been soaked in sample solution but might have grown roots if they had. In addition to the efficacy related to direct enhancement of root formation, 5,6-Cl\(^{-2}\)-IAA may effectively permeate the hypocotyl and/or travel rapidly once inside the plant. 4-Cl-IAA also had strong rooting-promoting activity in that the induced root number was four times more than that of IBA at the same concentration. The dry weight of roots induced by 5,6-Cl\(^{-2}\)-IAA and 4-Cl-IAA was 1.8 times that of those induced by IBA. The smaller increase in dry root weight relative to rooting activity was not statistically significant.

Table 1. Root Formation-Promoting Activities of 5,6-Cl\(^{-2}\)-IAA, 4-Cl-IAA, IBA and IAA in Black Gram Cuttings

<table>
<thead>
<tr>
<th>Compound</th>
<th>Root number</th>
<th>Ratio to IBA (%)</th>
<th>Dry weight of roots (mg)</th>
<th>Ratio to IBA (%)</th>
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<tr>
<td>5,6-Cl(^{-2})-IAA (1)</td>
<td>157.2±12.5</td>
<td>1526±121</td>
<td>7.4±0.5</td>
<td>176±12</td>
</tr>
<tr>
<td>4-Cl-IAA (2)</td>
<td>42.7±3.2</td>
<td>415±31</td>
<td>7.6±0.5</td>
<td>181±12</td>
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<tr>
<td>IBA(^6)</td>
<td>10.3±1.2</td>
<td>100±12</td>
<td>4.2±0.4</td>
<td>100±10</td>
</tr>
<tr>
<td>IAA(^6)</td>
<td>6.0±0.4</td>
<td>58±24</td>
<td>3.7±0.3</td>
<td>88±7</td>
</tr>
<tr>
<td>Control</td>
<td>5.7±0.4</td>
<td>55±24</td>
<td>3.3±0.3</td>
<td>79±7</td>
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\(^{a}\) All compounds were used at 5×10\(^{-5}\) M. IBA was used as a standard promoter of root formation. Each value is presented as the mean±standard error of the mean, n=5. \(^{b}\) Dry weight of roots was measured after roots were air-dried at 25°C for two weeks. Each value is presented as the mean±standard error of the mean, n=5. \(^{c}\) IBA, 4-(3-indole)butyric acid. \(^{d}\) IAA, indole-3-acetic acid.
showed that the limited nutrients within the cuttings could be used to form large numbers of roots but that the growth of these roots could not be sustained without additional nutrients in the solution. At lower concentrations of these promoters, the growth and dry weights of roots increased (data not shown). The increased number and dry weight of roots by the promoters is very important for the production of healthy transplants during reforestation. 5,6-Cl₂-IAA is the most potent of the known natural and synthetic rooting promoters while 4-Cl-IAA is the most potent of all known natural promoters. These two compounds will be the most appropriate candidates for active ingredients in new strong rooting promoters.²³⁻²⁸

In the estrogen receptor binding assay, neither 5,6-Cl₂-IAA nor 4-Cl-IAA had estrogenic activity at any concentration tested, although many chlorinated pesticides do have endocrine-disrupting activity (Fig. 3).⁷⁻⁹ On the other hand, bisphenol A, a control compound, showed apparent estrogenic activity at concentrations greater than 1×10⁻⁴ M, although this activity was much weaker than that of 17β-estradiol. These results suggest that 5,6-Cl₂-IAA and 4-Cl-IAA as active ingredients of new rooting promoters will have the added benefit of not disrupting endocrine activity. Further, this result demonstrates the safety of edible Viciae plants, the immature seeds of which contain 4-Cl-IAA without exception. We are now continuing to improve rooting promoters using these compounds and testing root induction in trees that are usually at least partly resistant to this process.

References

23) M. Mitsuhashi, H. Shibaoka and M. Shimokoriyama: Plant Cell...


ピーキー濃度で検出されるものがある。

アルキレン架橋ビスイミダクロブリド誘導体の植物浸透移行による殺虫活性
森 勝，菊池真美，大野育也，利部伸三
アルキレン架橋ビスイミダクロブリド誘導体（Bis-IMI）の、シャーレ試験、葉面散布、イネ幼苗浸没処理及び浸透移行性実験条件における殺虫活性を求めた。シャーレ試験におけるモダカアブラムシに対する殺虫活性は架橋の長さによって変動し、ヘプタ（C7）およびオクタメチレン（C8）誘導体が最も高く、10 mg L⁻¹で高い殺虫活性を示した。同濃度の薬剤をキャベツへ葉面散布し、11日目に散布し12時間後に観察したところ、ヘキサメチレンC6およびC7誘導体はほぼ完全にモダカアブラムシを防除した。しかし、同様な条件下、モリプロトンカ（イネ）とアガ（キャベツ）に対する殺虫試験を行なったところ、2化合物の防除効果は弱かった。一方、イネ幼苗の根部浸没処理及びキャベツ幼苗への株元灌漑処理後、3，7，11日後に散布し12時間後に観察したところ、C7誘導体はモリプロトンカとモダカアブラムシに対して、C6誘導体はモリプロトンカに対して高い防除活性を示し、これらの化合物の浸透移行性が確認された。

Bis-IMI誘導体は、分子量、Log P 値、水素結合生成性原子数および自由回転結合数において、パラルクスクリーニングシステムによる殺虫性分子のための基準値からはれている。このような新しい骨格の化合物の殺虫特徴は、既存殺虫剤のデータを基にして誘導された判断基準だけでは説明できず、新たな構造因子を含めた考察が必要と思われる。

短 報
土によるテルプメトンとイソプロトンの吸着：Ca²⁺とK⁺カチオンの影響
Achouak El Arfaoui, Stephanie Boudesocque, Stephanie Sayen, Emmanuel Guillon
塩（KClとCaCl₂）が、テルプメトンとイソプロトンの